

1 Soil carbon mineralization as affected by water content and 2 nitrogen rate after ryegrass incorporated into soil

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10 Abstract

11
12 lanting cover crop has been suggested as a way of increasing soil organic carbon in agricultural land.
13 Ryegrass (*Lolium multiflorum* L.), as a cover crop, could improve soil fertility and lower soil CO₂
14 emission. However, effects of soil water content and nitrogen on soil carbon mineralization after
15 ryegrass incorporation are not fully understood. The present study was to investigate the effect of soil
16 water content and nitrogen rate on soil carbon mineralization after ryegrass incorporated into upland
17 red soil (Ferralsols). A laboratory experiment was established, including soil water contents [15%
18 (W1), 30% (W2), 45% (W3)] and nitrogen rates [0 (N1), 60 mg/kg(N2), 120 mg/kg(N3)]. The results
19 showed that the highest soil carbon mineralization accumulation was observed in W3N3. Nitrogen
20 application inhibited carbon mineralization rate and accumulation in the late stage of ryegrass
21 incorporation at W1, but increased carbon mineralization rate and accumulation at W2. With increasing
22 soil water content, nitrogen application could improve soil carbon mineralization at the early stage of
23 ryegrass incorporation. In conclusion, soil nitrogen and water content could regulate soil carbon
24 mineralization. Considering to reduce the soil CO₂ emissions, rational nitrogen application should be
25 taken seriously during cover crop (ryegrass) incorporated into the upland red soil.

26 **Key words:** Ferralsols, carbon mineralization rate, accumulation, first-order kinetic
27 equation

28 1 INTRUDUCTION

29
30 oil organic carbon has a significant important role in the global carbon cycle(Bailey et al.
31 2018). Soil carbon decomposition and sequestration is mainly affected by cropping
32 systems(Moreno-Cornejo et al. 2015), tillage methods, cover crops(Poeplau and Don 2015),
33 organic manure and chemical fertilizer(Lehmann and Kleber 2015) in field. Soil carbon
34 mineralization is limited by environmental factors, such as field moisture capacity(O'Connell
35 et al. 2016), temperature(Giardina and Ryan 2000), climate(Luo et al. 2017), etc. Soil water
36 content impacts soil oxygen, which would inhibit soil microbial activity, and finally affected
37 soil carbon mineralization(Rivas et al. 2014). The influences of exogenous nitrogen input on
38 soil carbon mineralization varied with environmental factors(Coucheney et al. 2013). The
39 major objects of present documents are forest (Fisk et al. 2015), wetland(Zheng et al. 2018),
40 grassland(Poeplau et al. 2017) and cropland(Das et al. 2019), but there are few reports about
41 effects of soil water content and nitrogen rate on soil carbon mineralization after cover crop
42 incorporated into red soil.

43
44 red soil is the third most important soils of the world covering 13% of the land area(Baligar et
45 al. 2004). Meanwhile, red soil is an important land resource in south China because of the
46 great potential for agricultural production. However, there are some problems remained, such
47 as uneven distribution of precipitation in space-time, serious soil and water loss, land
48 degradation, excessive fertilizer application and so on(Zhang et al. 2013). There are highly
49 leached and serious acidification, and have a low organic matter and nutritional
50 deficiencies(Wilson et al. 2004). Whereas, the red soil region belongs to subtropical areas in
51 China, which has abundance of natural resources, such as light, temperature and water, for
52 planting cover crops in winter.

53

over crops had the ability to scavenge nutrients, and thus can decrease nitrate leaching(Carey et al. 2018), alleviate soil erosion(Gómez et al. 2018) and improve soil organic matter(Wolff et al. 2018). Ryegrass, as a forage grass or cover crop, has high cold resistance, high biomass production, and rich nutritional value. Sowing ryegrass could mitigate nitrous oxide emissions(Pilecco et al. 2019) and remediate contaminated soil(Li et al. 2020). Ryegrass incorporation could improve crop yield and soil quality in paddy field(Yang 1996), ryegrass residues could also lower soil CO₂ emissions and increase soil C stocks(Mwafulirwa et al. 2019). Soil carbon mineralization is an important indicator for measuring soil quality(Gil-Sotres et al. 2005). Planting ryegrass as cover crop after sweet corn harvested in late October, and incorporated into soil in next early April, was it beneficial to improve soil quality? Our results showed that applied chemical nitrogen fertilizer (60 mg/kg) could inhibit ryegrass decomposition and nitrogen release(Yang et al. 2018), and soil nitrogen mineralization(Wang et al. 2018) at the early stage of ryegrass incorporation, which might alleviate the loss of soil nitrogen. However, effect of soil water content and nitrogen rate on soil carbon mineralization after ryegrass incorporation is not clear yet.

he objectives of this laboratory experiment were to study the influence of soil water content and nitrogen rate on soil carbon mineralization, and optimize soil water and nitrogen management under ryegrass incorporated into upland red soil. We hypothesized that soil water content and nitrogen rate could affect the ryegrass decomposition, and then regulate soil carbon mineralization in upland red soil.

2. MATERIALS AND METHODS

2.1 Ryegrass and soil

he ryegrass (*Lolium perenne* L.cv. 'Ganxuan No.1') was planted after sweet corn harvested on the 15th October, 2013, and harvested before sowing sweet corn on 1st April, 2014. The ryegrass was cut into small pieces and dried in an oven at 105 °C for 30 min, and then at 80 °C until a constant weight was reached. The main nutritional components of dry ryegrass were tested: total C 360.19 g/kg, total N 27.62 g/kg, C/N 13.04, total P 21.08 g/kg and total K 67.17 g/kg.

he experimental soil, a Ferralsols, was collected from 0-20 cm layer from Jiangxi Agricultural University Sci-tech Park in Nanchang, China (28° 45' N, 115°50' E). The soil was air-dried, removed visible plant roots, passed through a 2-mm sieve for the incubation study. The basic chemical properties of the soil were shown in Table 1.

Table 1. Soil chemical characteristics

Soil type	pH	Soil Organic C (g/kg)	Total N (g/kg)	Available N (mg/kg)	NH ₄ ⁺ -N (mg/kg)	NO ₃ ⁻ -N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Original soil	4.85	8.59	1.47	100.23	33.15	30.44	65.98	152.75
Incubated soil	4.65	11.01	2.05	136.17	42.53	31.49	106.19	208.41

2.2 Soil incubation

he experiment was a completely randomized design with three replicates which conducted from 11th May, 2014 to 8th August, 2014. There were 3 soil water contents [15% (W1), 30% (W2), 45% (W3)] and 3 nitrogen rates [0 (N1), 60 mg/kg (N2), 120 mg/kg (N3)]. 2.5 g dry ryegrass mixed into each 100 g soil, which was used as incubation soil. Each jar (250 ml) was packaged with 50 g incubation soil. The jars were added 7.5, 15 and 22.5 ml deionized water at W1, W2 and W3, respectively; and added 0, 14.15 and 28.30 mg ammonium sulfate ((NH₄)₂SO₄) at N1, N2 and N3, respectively.

2.3 The process of soil organic carbon mineralization

he experiment was measured potentially mineralization Carbon using soil CO₂ flux as described by(Rabbi et al. 2014). A centrifuge tube (10 ml) after adding 5 ml of 1 N NaOH was

placed in each jar, and the jars were sealed (air-tight) with wax, then shaded cultivation at 25 °C. The tubes were collected in 1, 3, 7, 13, 21, 31, 43, 57, 73 and 91 days. The amount of CO₂-C produced during incubation was trapped in 1 M NaOH. At first, added 1 ml saturated BaCl₂ and phenolphthalein indicator into the tube. And then the amount of CO₂-C was determined by titration against 0.1 M HCl with acid burette.

109 2.4 Parameter simulation and data analyses

his experiment used first-order kinetics model to assess soil carbon mineralization process (Cooper et al. 2011):

$$113 \quad Ct = C_0 \times (1 - e^{-kt})$$

The first-order kinetic model was simulated by Matlab7.0. Where C_t -total organic carbon at time t ; C_0 -the amount of the potential soil carbon mineralization; K -the decay constants of soil carbon mineralization rate, and t - the incubation time.

117 Soil carbon mineralization rate ($\text{mg}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) = Total soil carbon mineralization / days of
 118 incubation

Actual carbon mineralization rate (%) = Total soil carbon mineralization / Soil carbon content in incubated soil × 100%

Potential carbon mineralization rate (%) = Total potential soil carbon mineralization / Soil carbon content in incubated soil × 100%

123 Net carbon mineralization ($\text{mg} \cdot \text{g}^{-1}$) = Total soil carbon mineralization of nitrogen treatment
124 - Total soil carbon mineralization without nitrogen treatment

125 Net carbon mineralization rate (%) = Net carbon mineralization / Soil carbon content in
126 incubated soil × 100%

127 The statistical significance of differences among treatments were tested with ANOVA
128 (SPSS 19.0, SPSS Inc., USA), Duncan test was used to compare the means, differences at $p <$
129 0.05 level were considered to be statistically significant.

3 RESULTS

131 3.1 Dynamic of soil carbon mineralization accumulation

oil carbon mineralization was influenced by nitrogen rate and soil water content. At W1, compared to N1, the soil carbon mineralization contents under N2 and N3 were significantly reduced from the 7th to 91st day, the soil carbon mineralization contents under N2 and N3 were decreased by 13.57% and 12.24% in the 91st day, respectively. However, compared to N1, N3 significantly increased the soil carbon mineralization contents at W2 and W3; the soil carbon mineralization contents were increased by 6.85% and 9.89% at W2 and W3 in the 91st day, respectively. There was no significant difference between N2 and N3, the highest soil carbon mineralization contents was W3N3, reached to 4.14 mg/kg (Figure 1).

Different soil water contents had different influence on the soil carbon mineralization under the same nitrogen rate. Under N1, compared with W1, the soil carbon mineralization content in W2 was drastically reduced at the 1st and 3rd day, and significantly reduced at the 31st and 43rd day in W3, but significantly increased at the 13th day. Under N2 and N3, compared with W1, adding soil water content (W2 and W3) significantly increased soil carbon mineralization appeared in the middle and later periods (7th-91st day), there was no significant difference between W2 and W3.

149 Figure 1. Dynamic of soil carbon mineralization

150 Note: The W1, W2 and W3 represent the soil water contents 15%, 30% and 45%, respectively. The N1,
 151 N2 and N3 represent the nitrogen rates 0, 60 and 120 mg/kg, respectively. Error bars indicate the
 152 standard error of the mean ($n=3$), activities with different lowercase letters at the same column indicate
 153 significant differences at $P<0.05$.

Two-factor variance analysis indicated that nitrogen application and soil water content both significantly affected soil carbon mineralization (Table 2). With the same water content (W1), compared to N1, increasing nitrogen fertilization (N2 and N3) significantly inhibited soil

159 carbon mineralization amount and rate. However, there is no obviously difference between N2
 160 and N3. Under W2 and W3, compared to N1, increasing nitrogen fertilization (N3)
 161 significantly increased soil carbon mineralization amount and rate. Meanwhile, the soil
 162 carbon mineralization amount at N3 significantly increased than that at N2. With the same
 163 nitrogen rate (N1), compared to W1, increasing soil water content (W2 and W3) didn't
 164 significantly affect soil carbon mineralization amount and rate. At N2 and N3, compared to
 165 W1, soil water content (W2 and W3) significantly increased soil carbon mineralization
 166 amount and rate. Meanwhile, soil carbon mineralization amount and rate under W3 obviously
 167 improved than that under W2.

168 et carbon mineralization amount and rate at N2 and N3 both had negative value under W1.
 169 Under W2 and W3, net carbon mineralization amount and rate were significantly higher at N3
 170 than that at N2. With the same nitrogen rate (N1), net carbon mineralization amount and rate
 171 hadn't significant difference among W1, W2 and W3.

173
 174 Table 2. Effect of soil water content and nitrogen rate on accumulation and rate of soil carbon
 175 mineralization

Factors	Treatments	Accumulation mineralization amount (mg/g)	Mineralization rate (%)	Net mineralization amount (mg/g)	Net mineralization rate (%)
Nitrogen rate and Soil water content	W1N1	3.75±0.02cd	21.50±0.12b	-	-
	W1N2	3.24±0.04e	18.58±0.21d	-0.51±0.06d	-2.92±0.32d
	W1N3	3.29±0.06e	18.86±0.33cd	-0.46±0.05d	-2.63±0.31d
	W2N1	3.72±0.05d	18.62±0.33d	-	-
	W2N2	3.80±0.02cd	19.17±0.26cd	0.10±0.05bc	0.55±0.31bc
	W2N3	3.98±0.03b	19.58±0.22c	0.17±0.06a	0.96±0.35a
	W3N1	3.76±0.08cd	21.60±0.46b	-	-
	W3N2	3.89±0.07bc	22.30±0.43b	0.12±0.03bc	0.70±0.14bc
	W3N3	4.14±0.02a	23.74±0.10a	0.37±0.07a	2.14±0.39a
Two-factor variance analysis (<i>F</i> value)	Nitrogen rate	4.769*	-	6.417**	-
	Soil water content	115.147**	-	103.013**	-
	Nitrogen rate × Soil water content	20.271**	-	27.270**	-

176 note: Activities with different lowercase letters at the same column indicate significant differences at
 177 $p<0.05$. * indicate significant differences at $p<0.05$, ** indicate extremely significant differences at
 178 $p<0.01$.

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182 3.2 Dynamic of soil carbon mineralization rate

183 All the treatments had the maximum rate of soil carbon mineralization after 1st day of
 184 incubation and then dropped rapidly until the 91st day (Figure 2). soil carbon mineralization
 185 rates of N2 and N3 were significantly higher than that of N1 at W2. While at W3, compared
 186 with N1, soil carbon mineralization rate of N2 was decreased by 7.26%, but that of N3 was
 187 increased by 5.73%. In the N1, compared to W1, soil carbon mineralization rate of W2 was
 188 significantly decreased; but soil carbon mineralization rate of W3 was obvious higher than
 189 that of W2. In the N2 and N3, there was no significant difference in soil carbon mineralization
 190 rate among W1, W2 and W3. Soil carbon mineralization rate of W2 was significantly higher
 191 than that of W3 at N2. From the analysis results of Table 3, nitrogen rate and soil water
 192 content had significantly influenced on soil carbon mineralization rate.

193

194 Figure 2. Dynamic of soil carbon mineralization rate

195 Note: The W1, W2 and W3 represent the soil water contents 15%, 30% and 45%, respectively. The N1,

196 N2 and N3 represent the nitrogen rates 0 mg/kg, 60 mg/kg and 120 mg/kg, respectively. Error bars
 197 indicate the standard error of the mean ($n=3$), activities with different lowercase letters at the same
 198 column indicate significant differences at $P<0.05$.

199

200

Table 3. Two-way analysis of variance of soil carbon mineralization rate

Incubated days	Nitrogen rate	Soil water content	Nitrogen rate×Soil water content
1	12.93**	0.173	14.511**
3	8.956**	1.232	7.689**
7	8.049**	36.026**	7.313**
13	4.704*	111.088**	16.902**
21	0.688	71.872**	22.200**
31	2.917	57.272**	26.136**
43	7.791**	44.554**	33.159**
57	5.257*	99.258**	28.477**
73	5.099*	63.008**	16.726**
91	8.431**	92.652**	24.139**

201

202 note: * indicate significant differences at $p<0.05$, ** indicate extremely significant differences at $p<0.01$.

203

204 3.3 Kinetics parameters of soil carbon mineralization

205

T

206 he pattern of carbon mineralization curve fitted well in first order kinetics model ($R^2=0.97-$
 207 0.99) (Table 4). From all the treatments, the highest potential mineralization amount was
 208 observed in W2N3, and the lowest one was recorded in W1N2. With the same nitrogen rate
 209 (N1, N2, N3), compared to W1, W2 significantly increased the potential mineralization
 210 amount C0. However, There were no obvious difference between W2 and W3 at N2 and N3.
 211 Adding soil water content and nitrogen rate appropriately could increase the potential
 212 mineralization carbon in the red soil during ryegrass incorporation.

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N

214 itrogen rate and soil water content both obviously impacted on K value. The K value of
 215 W3N3 was significantly higher than other treatments. The K value of each treatment at W2
 216 was significantly lower than those at W1 and W3. The maximum of potential mineralization
 217 rate was obtained from W2N3, and the minimum one obtained from was W1N2.

218

219

Table 4. Kinetics parameters of soil carbon mineralization

Treatments	Fitting parameters			R^2	Potential mineralization rate (%)
	Potential mineralization amount C_0 (mg/g)	Mineralization rate constant $K(d^{-1})$			
W1N1	3.89±0.17bcd	0.0271±0.0026b	0.9915	22.33±0.96cde	
W1N2	3.38±0.06e	0.0267±0.0004b	0.9903	19.39±0.33f	
W1N3	3.56±0.09de	0.0241±0.0005b	0.9884	20.41±0.53ef	
W2N1	4.34±0.19a	0.0151±0.0009c	0.9928	24.8±1.14ab	
W2N2	4.23±0.09ab	0.0168±0.0006c	0.9927	24.28±0.52abc	
W2N3	4.49±0.03a	0.0155±0.0005c	0.9897	25.75±0.18a	
W3N1	3.97±0.08bc	0.0246±0.0002b	0.9789	22.76±0.46bcd	
W3N2	3.93±0.10bc	0.0277±0.0012b	0.9843	22.58±0.55cd	
W3N3	3.62±0.12cde	0.0362±0.0015a	0.9792	20.75±0.68def	
Nitrogen rate	3.183	4.82*	-	3.01	
Soil water content	34.648**	108.878**	-	32.775**	
	3.246*	11.673**	-	3.313*	

220

N

221 ote: Activities with different lowercase letters at the same column indicate significant differences at

222 $p<0.05$. * indicate significant differences at $p<0.05$, ** indicate extremely significant differences at
 223 $p<0.01$.

224

225 **4 DISCUSSION**

226 The present work indicated exogenous nitrogen addition inhibited soil carbon
 227 mineralization under low soil water content (15%). Soil microorganisms would be given
 228 priority to aerobic respiration, decomposed small molecule matter, enhanced microbial
 229 activity, and accelerated the mineralization efficiency(Ross et al. 2013). Low soil water
 230 content might inhibit the soil enzyme activity(Marschner and Kalbitz 2003) and microbial
 231 activity(Linn and Doran 1984), meanwhile, overuse mineral N could increase the aromaticity
 232 and or complexity of DOM molecules(Michel et al. 2006) , those might mitigate the
 233 mineralization of soil organic matter.

234 Increasing soil water contents (30 and 45%) with nitrogen fertilizer application could
 235 improve soil carbon mineralization in the present results. The similar results were reported in
 236 wheat-maize cropping system(Kan et al. 2020), floodplain wetlands(Yin et al. 2019) and
 237 Wuyi mountains(Xu et al. 2019). Increasing nitrogen fertilization could improve soil carbon
 238 mineralization during ryegrass incorporation with the soil water contents (30 and 45%). The
 239 reasonable soil moisture could increase microbial activity(Linn and Doran 1984), and
 240 improve rates of aerobic heterotrophic respiration(Das et al. 2019), then increase soil carbon
 241 mineralizability. Nitrogen fertilizer could increase soil carbon mineralization compared to
 242 without nitrogen fertilizer(Wang et al. 2020). The initial N concentration of the residues
 243 exerted a great influence on C mineralization. High N content supplied available N to soil
 244 microorganisms in short term and stimulate microbial activity(Ding et al. 2018). Residue with
 245 high N concentrations and low C/N ratio could accelerate the initial C mineralization(Raiesi
 246 2006), those were benefit to soil organic carbon mineralization.

247 The high soil carbon mineralization rates were all observed in the early stage (the first 7
 248 days) after ryegrass incorporation. This might be sufficient carbon and nitrogen resource in
 249 soil and ryegrass was rapidly degraded, that provided the fundamental materials for the
 250 reproducing of soil microorganisms during the early period. In the present work, nitrogen
 251 application inhibited the rate of soil carbon mineralization at low soil water content. The soil
 252 enzyme activity is a key factor in the decomposition of soil organic matter, it might limit by
 253 low soil water content(Marschner and Kalbitz 2003). As the soil water content increasing,
 254 adding nitrogen fertilizer significantly improved the soil carbon mineralization rate. This
 255 result was consistent with Li et al(2014). Previous studies found that increasing water content
 256 could improve soil carbon mineralization rate with 45% to 75% upland field capacity(Norton
 257 et al. 2012). Reasonable soil water content might promote the soil microbial and enzymatic
 258 activities(Galantini and Rosell 2006), and nitrogen fertilizer supply more available N(Ding et
 259 al. 2018), both leading to higher rate of soil carbon mineralization.

260 In our incubation experiment, the first-order kinetic equations were better fit SOC
 261 mineralization processes. it was similar with previous report(Yin et al. 2019). In the present
 262 work, soil water content significantly influenced the potential carbon mineralization. Potential
 263 mineralizable C (C_0) determined from the equation which is a measure of active or labile and
 264 easily decomposable SOC. These parameters might be used to determine the effect of
 265 agricultural practice and tillage on C sequestration and short term nutrient turn over or
 266 fertility(Doyle et al. 2004).

267 **5 CONCLUSIONS**

268 Nitrogen application significantly inhibited the soil carbon mineralization in the late stage
 269 of ryegrass incorporation at 15% soil water content, but increased the carbon mineralization at
 270 30% soil water content. Without nitrogen application, increasing soil water content
 271 significantly inhibited the soil carbon mineralization at the early stage of ryegrass
 272 incorporation. However, soil water content increased the soil carbon mineralization with
 273 nitrogen applied. In a conclusion, nitrogen fertilizer application and soil water content
 274 significantly affected the soil carbon mineralization after ryegrass incorporated into red soil.
 275 In order to improve soil carbon sequestration and reduce greenhouse gas emission, rational
 276 nitrogen application should be taken seriously during cover crop (ryegrass) incorporated into

277 the upland red soil.

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282 **Conflicts of Interest:** The authors declare no conflict of interest.

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